Genetic population structure of harbour porpoises (*Phocoena phocoena*) in the North Sea and Norwegian waters

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ABSTRACT

The harbour porpoise (*Phocoena phocoena*) is subject to a high rate of incidental mortality in fisheries worldwide and, in some areas, these rates are sufficiently high to warrant concern over population sustainability. Thus, the definition of sub-populations is paramount to the conservation of this species. To investigate the population structure in northeastern Atlantic waters, genetic sequence variation in mitochondrial DNA was examined in porpoises incidentally bycaught or stranded. The first 200 base-pairs of the control region were sequenced in 36 females and 47 males from Norwegian waters of the Barents and North Seas. In addition, 35 females and 31 males from United Kingdom waters, sequenced in a previous study (Walton, 1997) were included as a third study group. One haplotype was found to be common in all geographic groups, accounting for over 49% of all individuals sequenced. An analysis of molecular variance showed no significant difference among males from these regions. However, females showed a greater degree of genetic differentiation for both haplotype frequencies (F_{ST}) and molecular diversity (Φ_{ST}) than males. There was a significant difference ($\alpha = 0.05$) in the haplotype frequencies between the Barents Sea and North Sea UK female porpoises when adjusted for multiple comparisons. Haplotype frequencies showed a significant difference between the North Sea UK and North Sea Norway females only after porpoises from the Shetland Islands were excluded from the North Sea UK sample. A phylogenetic tree revealed two main haplotypic clades, although there was little geographic structuring among these clades. These results are consistent with findings from other areas and suggest females are more philopatric than males. In spite of the lack of significant phylogenetic structuring, differing haplotype frequencies suggest that the North Sea UK and the Barents Sea sub-populations should be considered separate management units. In addition, haplotype frequency differences among the North Sea Norway and North Sea UK females (excluding Shetlands) also suggest the presence of separate management units within the North Sea.

KEYWORDS: HARBOUR PORPOISE; STOCK IDENTITY; GENETICS; CONSERVATION; INCIDENTAL CATCHES; MANAGEMENT

INTRODUCTION

Cetaceans, and in particular the harbour porpoise (*Phocoena phocoena*), are vulnerable to interactions with both bottom set-net and surface driftnet fisheries (Perrin *et al.*, 1994). It is thought that current rates of removal through bycatch in some areas are too high to allow them to remain viable (Woodley and Read, 1991; Jefferson and Curry, 1994; Woodley, 1995; IWC, 1996). In addition, the harbour porpoise is virtually absent in some areas of historical occurrence such as the English Channel and the Baltic Sea (Jefferson and Curry, 1994; Berggren and Arrhenius, 1995). Although the reasons for the harbour porpoise's decline in these areas are not well documented, bycatch and the historical direct hunt in the Danish Belt Seas (Berggren and Arrhenius, 1995) could have contributed. Such incidences may be a warning that the species may be vulnerable to local extinction. Alternatively, distribution and/or migration patterns could be subject to local fluctuations leading to the perception that abundance has decreased (Teilmann and Lowry, 1996).

Given this vulnerability, elucidation of the stock structure of the harbour porpoise is extremely important in the formation and implementation of conservation and management plans. However, in many areas of the harbour porpoise's range, the structure of sub-populations is still unknown or not well understood (Donovan and Bjørge, 1995). One such area that has been little studied is the coast of Norway. In this area, harbour porpoises have a contiguous distribution, extending from the Skagerrak Sea in the east, into the North Sea, and northwards into the Barents Sea (Fig. 1). Porpoises are year-round residents of the entire coastline, including fjords and large bays. It has been suggested that there are at least two sub-populations, or stocks, in these waters. Gaskin (1984) speculated, on the basis of oceanographic features, that porpoises in Norwegian waters may be divided into northern and southern sub-populations with the line of separation near Vestfjorden, a deep fjord near the southern tip of the Lofoten Islands (67°N). Later, the International Whaling Commission (IWC) tentatively suggested a possible division between northern and southern Norway at approximately 66°N, after noting a low offshore density of porpoises in that area (Bjørge and Øien, 1995; Donovan and Bjørge, 1995; IWC, 1996).

Genetic techniques have increasingly been used to elucidate population structure and, recently, the use of mitochondrial DNA (mtDNA) analysis has been useful in a variety of studies. The haploid state of mtDNA in concert with maternal inheritance reduces the effective population size to one quarter that of nuclear DNA, increasing the effect of genetic drift because drift proceeds at a greater rate when population sizes are small (Moritz, 1994). Additionally, rapid evolution of the molecule (Brown, 1983) allows for a rapid accumulation of differences between isolated groups.

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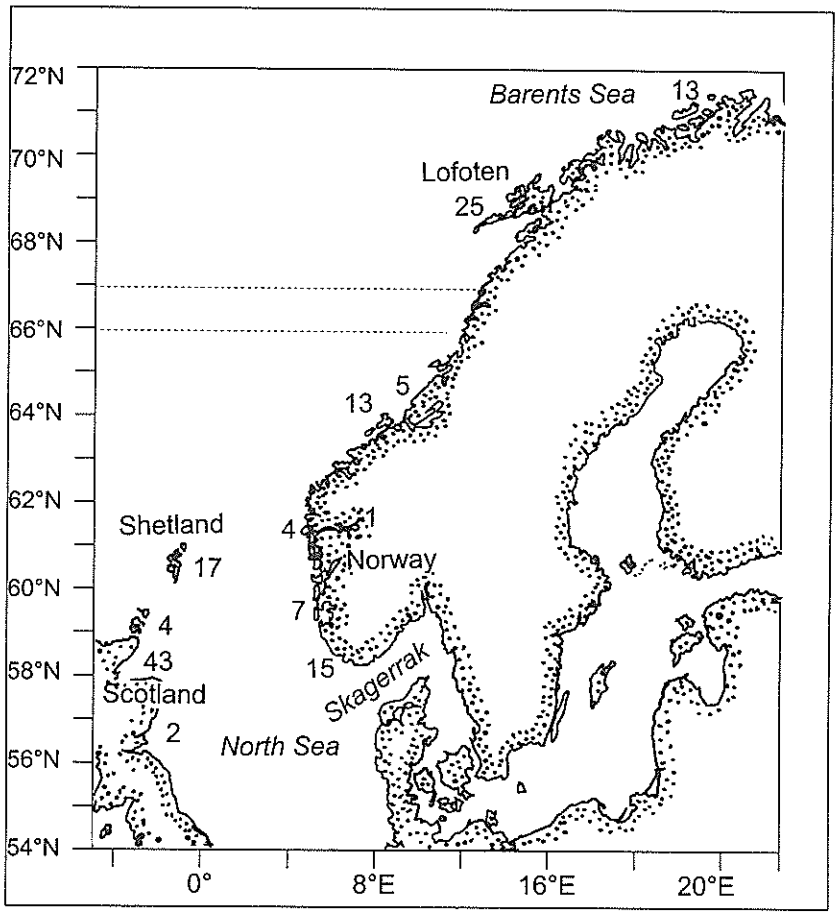

Fig. 1. Sampling sites and sample sizes of harbour porpoises from the Norwegian coast and northern UK waters. Barents Sea porpoises were collected north of 67°N, while North Sea Norway samples were collected along the Norwegian coast, south of 64.75°N. UK samples were collected from the coast of Scotland, the Orkney Islands and the Shetland Islands. All UK samples are a sub-sample of those found in Walton (1997). Latitudes 66°N and 67°N are indicated by dotted lines.

For example, using sequences from the control region of mtDNA, Rosel *et al.* (1995) found a high degree of genetic differentiation among porpoises from the North Pacific and North Atlantic, with no shared haplotypes among ocean basins. Therefore, these populations can be considered reproductively isolated, and constitute true allopatric populations. In spite of a lack of any obvious geographic barriers, significant genetic differences were also found among sub-populations within the North Pacific (Rosel *et al.*, 1995). Using restriction fragment length polymorphism (RFLP) analysis of mtDNA, Wang *et al.* (1996) found significant genetic differences among porpoises from Newfoundland, the Gulf of St Lawrence and the Bay of Fundy/Gulf of Maine. Differences were less apparent among males from these regions leading to the conclusion that females are more philopatric than males. Further, Rosel *et al.* (In press) found significant differences in mtDNA sequences among four regions in the western North Atlantic. However, no differences were found when six microsatellite loci were examined for the same samples (Rosel *et al.*, In press), further supporting the hypothesis that females are more philopatric than males, and that gene flow may be predominantly occurring through males.

Several genetic studies support the division of sub-populations in the eastern North Atlantic: Wang and Berggren (1997) concluded that porpoises from the Swedish Baltic Sea, Kattegatt/Skagerrak Seas and western Norway comprised three genetically distinct sub-populations based on mtDNA RFLP analysis; Tiedemann *et al.* (1996) concluded that the mtDNA control region haplotype frequencies of porpoises from the German Baltic Sea are distinct from porpoises in the North Sea; and Walton (1997) found there may be fine scale differences among porpoises from British waters. In the last study he found that females from northern UK North Sea waters differed from those in the southern part, and from those in the Irish Sea/Celtic Shelf. This proposed fine scale division of porpoises in the North Sea is in accordance with a study of allozymes in which it was concluded that the entire North Sea may consist of several different breeding stocks (Anderson, 1993).

Børjessen and Berggren (1997) found different morphological skull characteristics between the Baltic Sea and the Kattegat/Skagerrak Seas in females but not males. They suggested that these two areas contain separate sub-populations and that this supported the genetic findings of Wang and Berggren (1997). Additional information on stock structure can be obtained from studies of environmentally acquired factors. For example, Kleivane *et al.* (1995) found that PCB levels in male harbour porpoise blubber suggested ecological separation of porpoises from the Belt area of Denmark, the Norwegian North Sea and northern Norway. It is interesting to note that on an ecological time scale, these areas show distinct groups of males, whereas most genetic studies suggest that males form more widespread groups.

In this study, two models for the distribution of porpoises along the Norwegian coast and within the North Sea are considered:

(1) a parapatric sub-population model (spatially separate but adjoining) with no zoogeographic barriers, but with distinct genetic discontinuities; and
(2) a metapopulation model with no zoogeographic barriers and no distinct genetic discontinuity, but with genetic differences increasing with geographic distance.

Given the results of previous studies, the hypothesis that females are more philopatric than males and should show a greater degree of genetic differences in mtDNA is also considered. These hypotheses are examined by studying variations in the nucleotide sequence of a portion of the mtDNA control region of porpoises from three regions (Barents Sea, North Sea Norway and North Sea UK).

METHODS

Muscle samples (36 females, 47 males) were collected from bycaught porpoises obtained in the Norwegian coastal gillnet fishery between 1988 and 1990, and from strandings in 1997 and 1998 (one female, one male). The port of landing was the best estimate of geographic location available, and the porpoises were classified accordingly. Porpoises were grouped following the IWC suggestions (Donovan and Bjørge, 1995), i.e. animals collected south of 66°N were assigned to the North Sea Norway group and those collected north of 66°N to the Barents Sea group. Four porpoises caught between 65°N and 67°N were excluded from the analysis given the uncertainty regarding the 'border' in this area. To minimise potential seasonal variation, only porpoises collected from May-September were included in this study.

DNA was extracted from 70-100mg of frozen muscle using standard procedures, namely digestion with proteinase K followed by a phenol/chloroform extraction procedure (Sambrook *et al.*, 1989). The tissue was incubated in EDTA (0.2mol/l, pH = 8.0) and proteinase K (10mg/ml) overnight at 37°C. DNAase-free RNAase (10mg/ml) was then added to the solution and incubation continued for an additional hour. The contents were transferred to serum separation tubes and contaminants removed from the DNA by one phenol extraction followed by one phenol/chloroform extraction with a final chloroform extraction. DNA was precipitated from the aqueous layer with two volumes of 96% ethanol and one-tenth volume sodium acetate (3mol/l, pH = 6.8), followed by a 70% ethanol rinse. The ethanol was evaporated and the pellet was then redissolved and stored in TE buffer (pH = 8.0).

The first 350 base-pairs (bp) of the control region of the mitochondrial DNA molecule were amplified using the polymerase chain reaction (PCR; Saiki *et al.*, 1985) with primers Tp and CR1 (Rosel *et al.*, In press). The Tp primer targets a region in the proline tRNA gene upstream of the control region, while the CR1 primer targets a conserved central region of the control region. The cycling profile consisted of 25 cycles of 95°C for one minute, 55°C for one minute, and 72°C for one minute. The PCR products were purified by either gel purification (Sephaglas Band Prep Kit, Pharmacia Biotech, Buckinghamshire, England) or with the exonuclease pre-sequencing kit (Pharmacia Biotech). The PCR product was sequenced from the 5′ end with the Tp primer, using an ABI Prism dye-terminator cycle sequencing kit according to the manufacturers instructions (PE Biosystems, Foster City, CA, USA). Products were purified on Auto-Seq spin columns (Pharmacia Biotech), precipitated in a vacuum centrifuge and applied to a Perkin Elmer ABI Prism 377 automated DNA sequencer (SARS Center, University of Bergen).

In a previous study of geographic variation in mtDNA, Walton (1997) sequenced the first 200 bp of the control region of porpoises around the British Isles and these sequences from 35 females and 31 males collected in the North Sea off the coast of Scotland were assigned to the North Sea UK group for the present study. Given the nature of Walton's study, comparisons among groups in this paper have only been conducted on a consensus region for the first 200 bp.

An Analysis of Molecular Variance (AMOVA: Excoffier *et al.*, 1992) was used to estimate the degree of subdivision among geographic groups using the computer program Arlequin (Schneider *et al.*, 1997). The AMOVA procedure can calculate various analogues of Wright's Fixation Index F_{ST} (Wright, 1921) which is a measure of population, or group, subdivision. Monte Carlo resampling simulations with a minimum of five thousand permutations were then performed to estimate whether the value obtained from the data differed significantly from zero. The AMOVA was used to calculate two different estimators of population subdivision:

(1) conventional F-statistics (F_{ST}) based on haplotype frequency data, not taking into account the molecular diversity between pairs of sequences;
(2) Φ_{ST} based on both haplotype frequency information and the amount of genetic distance (or molecular diversity) among all unique haplotypes.

For the Φ_{ST} determination, the Tamura-Nei method with the gamma correction ($\alpha = 0.99$) was used to generate the distance matrix (Rosel *et al.*, 1995). This method is appropriate for calculating genetic distances from control region data as it can correct for rate variability among sites common to this region of DNA. Finally, the Bonferroni sequential correction for multiple comparisons (Rice, 1989) was used when evaluating the significance of the pairwise differences.

Haplotypic diversity, δ and nucleotide diversity, π (Nei, 1987) were estimated within each geographic group using the Arlequin software (Schneider *et al.*, 1997). Haplotypic diversity is a function of the number of different haplotypes and their frequencies in each group, while nucleotide diversity describes the average number of nucleotide differences between all pairs of haplotypes (Nei, 1987). The Tamura-Nei method and a gamma correction of $\alpha = 0.99$ were used when estimating nucleotide diversity (Rosel *et al.*, 1995). Genetic distance (d_A), or the net number of nucleotide substitutions between each of the putative sub-populations was also calculated according to Nei (1987).

The North Sea UK sample included porpoises from the Shetland Islands, which have an intermediate geographic position in the North Sea between Norway and Scotland. In order to minimise spatial variation in the groups, an *a posteriori* AMOVA was run with the Shetland porpoises excluded from the UK group (nine females and eight males). This AMOVA was run on females and males separately for the North Sea UK and North Sea Norway porpoises.

An estimate of the long-term average absolute number of migrants per generation (Nm) exchanged between groups was calculated as:

$$Nm \approx (1/F_{ST} - 1)/2$$

However, this estimate should be treated with caution as it is based on a number of assumptions which are easily violated in real populations. These assumptions include: sub-populations are of equal size, sub-populations are in migration-drift equilibrium and sub-populations are

exchanging equal numbers of migrants. In addition, a high standard error of F_{ST} will result in a high standard error in the estimation of Nm.

Relationships among haplotypes were estimated from an unrooted neighbour-joining tree (Saitou and Nei, 1987). The intention was to group haplotypes according to similarity, not to describe polarity or to infer evolutionary relationships, and thus, the tree was unrooted and no outgroup was used. The tree was produced using the MEGA software package (Kumar *et al.*, 1993) and genetic distances were estimated using the Tamura-Nei method (gamma correction, $\alpha = 0.99$). A minimum spanning network (Minspnet, L. Excoffier) was also constructed to better illustrate the relationships among haplotypes.

RESULTS

Haplotype diversity and nucleotide diversity by region and sex are given in Table 1, while genetic distances (d_A) between the groups are found in Table 2. A total of 27 unique haplotypes for the first 200 bp were found for porpoises from all regions (Table 3 and 4). For the six haplotypes found only in UK waters, the original haplotype names from Walton (1997) are retained (D, L, R, V, Y, AK). There was a single dominant haplotype (N1) which accounted for 49.5% of all individuals. This haplotype was dominant in each region for both sexes. Five haplotypes (N3, N4, N12, L, V) accounted for 28.5% of the variation while the remaining 22 haplotypes accounted for 22% of the variation. Of those 22 haplotypes, 11 were represented by a single individual. In the first 200 bp sequenced, 20 sites were variable and 10 were phylogenetically informative. Three of the bp changes were insertion/deletion events, and the remaining changes were all transitions. Haplotype frequencies for each geographic area are given in Table 4. Additional details regarding the genetic diversity indices of the North Sea UK porpoises can be found in Walton (1997).

Table 1

Haplotype and nucleotide diversity for the first 200 base pairs of the mtDNA control region in harbour porpoises from the Barents Sea (BS), North Sea Norway (NSN) and North Sea UK (NSUK).

	n	Haplotypic diversity (δ)	Nucleotide diversity (π)
Females BS	20	0.82	0.013
Females NSN	16	0.76	0.013
Females NSUK	35	0.64	0.010
Males BS	18	0.70	0.012
Males NSN	29	0.74	0.012
Males NSUK	31	0.77	0.012
Both Sexes BS	38	0.76	0.012
Both Sexes NSN	45	0.74	0.013
Both Sexes NSUK	66	0.71	0.012

Table 2

Percent genetic distance (d_A) between each of the putative subpopulations of harbour porpoises from the Barents Sea, North Sea Norway and North Sea UK. These values are corrected for within population variation and were estimated with gamma distances ($\alpha = 0.99$) by the Tamura-Nei model. Females are on the top half of the matrix and males on the bottom half.

	Barents Sea	North Sea Norway	North Sea UK
Barents Sea		0.00	0.07
North Sea Norway	0.00		0.07
North Sea UK	0.02	0.00	

Based on haplotype frequencies (F_{ST}), a significant difference was found between the North Sea UK and the Barents Sea for females ($p = 0.014$), even after correction for multiple comparisons ($\alpha = 0.05$). The number of female migrants (Nm) between these two regions was estimated at 5.6 per generation (Table 7). The difference between the North Sea UK and North Sea Norway females was not significant ($p = 0.051$). None of the population pairwise Φ_{ST} values based on molecular diversity were significantly different for females when adjusted for multiple comparisons (Table 5). No significant differences were found among males for genetic diversity or haplotype frequencies (Table 6).

The *a posteriori* AMOVA for the North Sea Norway and North Sea UK females (minus Shetland porpoises, see Table 4) showed significant differences for haplotype frequencies ($F_{ST} = 0.084$, $p = 0.030$) but not for haplotype plus molecular diversity ($\Phi_{ST} = 0.077$, $p = 0.064$). The number of female migrants between these two regions was estimated at 5.5 per generation. Thus, there was a significant difference between the UK and Norway for haplotype frequency when Shetland females were excluded from the UK group, but not when the Shetland females were included. There were no significant differences among males ($F_{ST} = 0.021$, $p = 0.129$; $\Phi_{ST} = 0.000$, $p = 0.532$) when the Shetland porpoises were excluded.

The neighbour-joining tree showed two main groupings of haplotypes (Fig. 2). 'Group A' contained the most common haplotype (N1) as well as several closely related haplotypes. All putative 'sub-populations' were approximately equally represented in Group A. All group B haplotypes differed from Group A by two transitions at sites 17 (A-G) and 50 (C-T). Transitions, or the absence of, at sites 64 (C-T) and 110 (G-A) divided Group B into four additional groups (B1-B4). Barents Sea porpoises were frequent in Group B1, but infrequent in Group B2, while North Sea UK porpoises were common in Group B4, but not in Group B1. Although this may indicate a slight degree of phylogeographic structuring between the Barents Sea and the North Sea UK, all haplotypes in Group B were closely related.

The minimum spanning network of all haplotypes showed further relationships among haplotypes that were not apparent from the phylogenetic tree (Fig. 3). Haplotype N1 and several closely related haplotypes showed a star-like phylogeny while the remainder of the haplotypes can be derived from N1 through haplotype N10. The complex network in these haplotypes is indicative of a higher degree of homoplasy among haplotypes not closely related to N1.

DISCUSSION

Molecular ecological studies are useful to conservation biology as a means of identifying biological units, as opposed to groups which are convenient for the purposes of management (Donovan, 1991). Biological units can take several forms, based on the amount of genetic diversity between them. Groups with unlimited gene flow between them would be considered completely panmictic. An initial separation between groups, whether due to a physical geographic separation or low gene flow for an extended amount of time, will be characterised by differing haplotype frequencies. This is because haplotype frequencies normally respond rapidly to initial separation or low gene flow (Avise, 1992; Moritz, 1994). These types of groups are usually termed 'management units' (MUs) and can be considered biologically meaningful, as they are genetically distinguishable (Moritz, 1994). Such groups will not show

Table 3

Twenty-one unique haplotypes (N1-N29) found for the mtDNA control region in porpoises from the Barents Sea and the North Sea, plus six unique haplotypes (D-AK) found only in the North Sea UK group[1]. Substitution site number is listed across the top, while haplotype numbers are listed in the column to the left. Total number of individuals for each haplotype are given (n). These haplotypes are cross-referenced against those from other studies of the mtDNA control region in harbour porpoises: NA=North Atlantic (Rosel *et al.*, 1995); UK=United Kingdom waters (Walton, 1997); G=German waters (Tiedemann *et al.*, 1996).

	n	1	2	6	17	18	42	50	52	55	64	66	67	80	85	96	105	110	112	115	121	125	135	158	196	NA	UK	G[2]
N1	73	A	A	G	A	A	G	C	A	A	C	C	G	G	T	T	A	G	T	T	T	C	T	T	C	2, 8, 10	A	I, VII
N2	2	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	C	.	.	.	.			
N3	7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.		AH	
N4	6	-	.	.	G	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
N6	1	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.			
N7	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.			
N8	2	-	.	.	G	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T			
N9	2	-	.	.	G	G	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
N10	1	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.		W	
N11	2	.	.	.	G	.	.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.		AI	
N12	8	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	C	
N15	1	.	.	A	G	.	.	T	.	.	T	.	.	.	.	.	.	A	C	.	.	.	.	.	.			
N16	1	-	-	.	G	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
N17	2	.	.	.	G	.	.	T	.	-	T	.	.	.	.	.	.	.	.	.	.	.	.	C	.			
N19	2	.	.	.	G	.	.	T	.	-	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.			
N20	2	.	.	.	G	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.		H	
N21	1	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	5		
N24	1	.	.	.	G	.	.	T	.	.	.	.	A	.	.	.	G	.	.	.	.	.	.	.	.			
N26	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T		E	II
N28	1	.	.	.	G	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
N29	1	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	7	AB	
D[2]	4	.	.	.	G	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T			
L[2]	16	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T			
R[2]	2	.	.	.	G	.	A	T	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.			
V[2]	5	.	.	.	G	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
Y[2]	1	.	.	A	.	.	.	T	.	.	T	.	.	.	.	.	.	A	.	.	.	T	.	.	.			
AK[2]	1	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.			

[1] These haplotypes were found in UK waters only (after Walton, 1997) but are included in the North Sea UK group for this study.
[2] Sequencing in Tiedemann *et al.* (1996) began at site 4 as compared to the present study.

substantial phylogenetic structuring as separation has not been long enough for stochastic lineage sorting to eliminate haplotypes common to both groups (Avise *et al.*, 1987; Avise, 1992). If the groups remain separate, further differentiation should eventually arise as some of the haplotypes shared amongst MUs will be eventually eliminated through stochastic processes.

Substantial phylogenetic differences among putative sub-populations may never be completely discrete as long as the time from initial separation is minimal, and/or a small amount of movement between the regions is occurring. Even for mtDNA, complete monophyly of haplotypes among geographic groups is expected to arise only after $4n$ generations (Avise *et al.*, 1984; Moritz, 1994). In harbour porpoises, lineage sorting leading to monophyly may have occurred among ocean basins, as Rosel *et al.* (1995) found no shared haplotypes among harbour porpoises from the North Pacific, North Atlantic and Black Sea. Harbour porpoises in these three areas are thought to be reproductively isolated from each other and therefore allopatric, and have been isolated long enough to produce the genetic results supporting three monophyletic groups.

Geographic variation in mtDNA

Genetic differences in this study were observed among females, but not among males. These differences were larger between groups with a wide geographic separation (i.e. Barents Sea and North Sea UK), and less apparent for groups which were geographically close (i.e. North Sea UK and North Sea Norway). In particular, haplotype frequencies (F_{ST}) were significantly different between Barents Sea females and North Sea UK females although there was no corresponding significant difference for molecular diversity plus haplotype frequencies (Φ_{ST}). The observed differences between these groups supports the establishment of separate management units for these two areas. The differing haplotype frequencies among these regions, but the lack of substantial phylogenetic structuring, suggest either sustained but minimal spatial movements between these regions, or a recent subdivision of a common ancestral population. Given the lack of substantial geographic barriers, it is unlikely that movements will be sufficiently low to separate these sub-populations into distinct monophyletic groups. However, due to differences in haplotype frequencies these groups are sufficiently subdivided to be termed distinct management units.

Although there was no significant difference initially found between the North Sea Norway and North Sea UK females, the difference among these two groups was significant after the Shetland porpoises had been excluded from the North Sea UK group. Thus, the indications are less conclusive with respect to regarding the separation of the North Sea UK and North Sea Norway as management units. When the Shetland porpoises were included in the North Sea UK group, the F_{ST} values were less than, but comparable, to the North Sea UK-Barents Sea, although the result was not statistically significant. There are several possible explanations for this. Clearly, there could be sufficient movement across the North Sea to homogenise the putative sub-populations in this region. Alternatively, there could be substantial gene flow between the regions via the Shetlands and in such a case, the geographic groups would be arranged

Table 4

Distribution and number of mtDNA control region haplotypes found in the eastern North Atlantic. Key: BS=Barents Sea; NSN=North Sea Norway; NSUK=North Sea UK. Six of the haplotypes were found only in the NSUK group and were originally described by Walton (1997). Haplotypes found in the Shetlands are included in parentheses in the NSUK column.

	Females			Males		
	BS	NSN	NSUK (Shetland)	BS	NSN	NSUK (Shetland)
N1	8	8	18 (6)	10	15	14 (4)
N2	0	1	0	1	0	0
N3	3	1	0	2	1	1
N4	3	2	0	0	1	0
N6	0	1	0	0	0	0
N7	0	0	0	1	0	0
N8	0	0	0	1	1	0
N9	1	0	0	1	0	0
N10	0	0	0	0	1	0
N11	1	1	0	0	0	0
N12	2	0	1	1	2	2
N15	0	0	0	0	1	0
N16	1	0	0	0	0	0
N17	0	1	0	1	0	0
N19	0	0	0	0	2	0
N20	0	1	0	0	1	0
N21	1	0	0	0	0	0
N24	0	0	0	0	1	0
N26	0	0	0	0	1	1 (1)
N28	0	0	0	0	1	0
N29	0	0	0	0	1	0
D[1]	0	0	3 (2)	0	0	1
L[1]	0	0	11 (1)	0	0	5
R[1]	0	0	0	0	0	2 (2)
V[1]	0	0	2	0	0	3
Y[1]	0	0	0	0	0	1
AK[1]	0	0	0	0	0	1
Total	20	16	35	18	29	31

Table 5

Inter-population comparisons for female harbour porpoises using two measures of genetic distance. F_{ST} values (based on haplotype frequencies only) are shown in the upper matrix and Φ_{ST} values (based on haplotype frequencies and sequence diversities) are shown in the lower matrix. The significance levels are based on 5,000 Monte Carlo permutations. The Bonferroni critical values for each of the pairwise comparisons (p_1, p_2, p_3) are shown in brackets. The result is significant when the sample p value is less than the Bonferroni critical value given in the same cell of the table.

	North Sea UK	Barents Sea	North Sea Norway
North Sea UK		0.083 $p = 0.014$ ($p_1 = 0.017$)	0.061 $p = 0.051$ ($p_2 = 0.025$)
Barents Sea	0.066 p = 0.054 ($p_1 = 0.017$)		0.000 $p = 0.798$ ($p_3 = 0.050$)
North Sea Norway	0.065 p = 0.081 ($p_2 = 0.025$)	0.00 p = 0.968 ($p_3 = 0.050$)	

Table 6

Inter-population comparisons for male harbour porpoises using two measures of genetic distance. F_{ST} values (based on haplotype frequencies only) are shown in the upper matrix and Φ_{ST} values (based on haplotype frequencies and sequence diversities) are shown in the lower matrix. The significance levels are based on 5,000 Monte Carlo permutations. The Bonferroni critical values for each of the pairwise comparisons (p_1, p_2, p_3) are shown in brackets. The result is significant when the sample p value is less than the Bonferroni critical value given in the same cell of the table.

	North Sea UK	Barents Sea	North Sea Norway
North Sea UK		0.007 ($p = 0.274$) $p_2 = 0.025$	0.007 ($p = 0.245$) $p_1 = 0.017$
Barents Sea	0.011 (p = 0.246) $p_1 = 0.017$		0.000 ($p = 0.884$) $p_3 = 0.050$
North Sea Norway	0.000 (p = 0.423) $p_2 = 0.025$	0.000 (p = 0.683) $p_3 = 0.050$	

on an east-west cline. Thus, including the central area of the hypothesised cline (Shetlands) in the UK sample may conceal potential differences between Norway and the UK. That the exclusion of the Shetland porpoises resulted in a significant difference between the North Sea Norway and the North Sea UK, lends credence to the cline hypothesis. Unfortunately, sample sizes are as yet too small to fully understand the status of the Shetlands as an avenue for movement within the North Sea.

No differences were found between the North Sea Norway and Barents Sea females, and there are again several possible explanations for this. First, there may be sufficient movement along the Norwegian coast to diminish genetic differences. Alternatively, differences among regions could have been masked by inclusion of porpoises from a large geographical area especially for the North Sea Norway

Table 7

Matrix of Nm values based on F_{ST} (upper matrix) and Φ_{ST} (lower matrix) for female harbour porpoises.

	North Sea UK	Barents Sea	North Sea Norway
North Sea UK		5.55	7.75
Barents Sea	7.05		Infinite
North Sea Norway	7.24	Infinite	

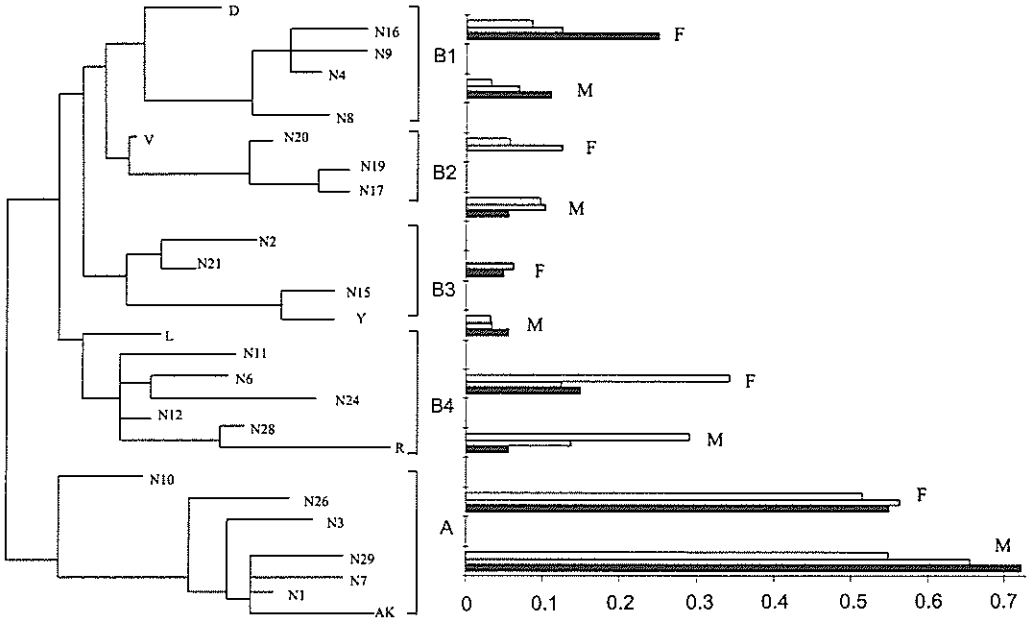

Fig. 2. A neighbour-joining tree of unique mtDNA control region haplotypes for harbour porpoises. Frequency distributions for each haplotype group (A, B1, B2, B3, B4) are shown for each gender (F-female; M-male) and geographic group (Barents Sea-black bars; North Sea Norway-grey bars; North Sea UK-white bars).

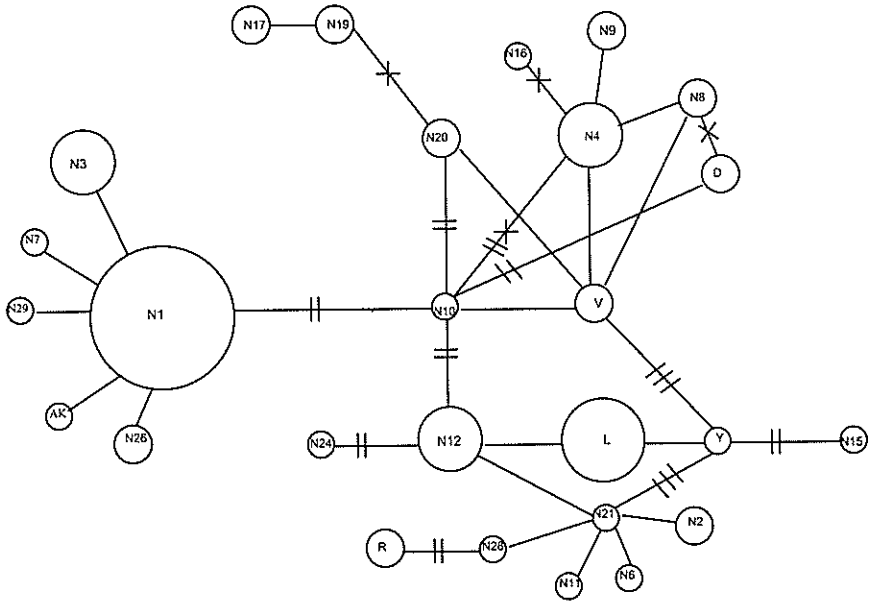

Fig. 3. A minimum spanning network showing the relationships between each of the haplotypes found in northeastern Atlantic harbour porpoises. All haplotypes are separated by at least one transition. Hatch marks on interconnecting branches indicate additional transitions between haplotypes. Crosses indicate insertion/deletion events. The approximate relative frequency at which each haplotype occurred is indicated by the size of the circles.

group. For example, sample size considerations led to porpoises ranging from 58-64.75°N being included in the same group. In such a large area, porpoises at the northern end of the North Sea Norway group could fall within an area of overlap between north and south. It seems likely that divergence through distance occurs, with porpoises from the extreme south coast genetically different from those along the extreme north coast. Thus, porpoises in the centre of the range could be in an area of sympatry, and their inclusion in one of the groups could mask possible differences in the ends of the range. The paucity of samples from the southernmost areas of the Norwegian North Sea made it necessary to include porpoises from a larger geographical area. Although porpoises from 65-67°N were not included in an effort to reduce spatial variation, a more strict sampling regime may help to resolve this uncertainty.

The statistical power of the tests applied here also warrants discussion. The low sample sizes, especially of females in the North Sea Norway group, mean that the statistical power to detect differences was low (Taylor *et al.*, 1997). The only way to increase power in this case is to increase sample sizes. Unfortunately, this is difficult, as all samples must be collected opportunistically from bycatch or strandings. These problems with sample size make it pertinent to ask what is the appropriate significance level for managers to consider when deciding on suitable

management units. If alpha levels are 0.05 but the observed significance between a set of putative sub-populations is just over that level, it may be prudent to give consideration to these sub-populations as management units. Indeed, in the report of a workshop on Molecular Genetics of Marine Mammals it was suggested that the alpha level should be increased from the customary 0.05 to 0.10 to avoid Type II errors, that is, falsely accepting the null hypothesis that two populations are identical (Dizon *et al.*, 1997).

Female philopatry and gene flow

Sub-population divergences indicated by haplotype frequencies (F_{ST}) for females were comparable to other areas which are thought to contain separate sub-populations (Rosel *et al.*, 1995; Walton, 1997). As in other mtDNA studies (Walton, 1997; Wang and Berggren, 1997) no significant differences were observed for any of the comparisons among males, providing additional support for the hypothesis that females are more philopatric than males, and males may occasionally move from their natal area into adjacent areas. Unfortunately, the aforementioned sampling difficulties have meant that most studies have combined samples from various local areas into a larger study group (e.g. North Sea Norway in this study). This makes determining the intensity of female philopatry problematic; for example, it is possible that females may form even more localised groups than our data and sampling regime are able to detect.

Female philopatry has been demonstrated in virtually every mtDNA study of the harbour porpoise to date, implying that gene flow among sub-populations occurs largely through males. However, because mtDNA is not passed on to the offspring through males, such an analysis provides information on male spatial organisation and not on the amount of gene flow occurring via males. Lack of differences in mtDNA among males implies that males may more readily move between areas, however this situation may decrease our ability to detect genetic differences among sub-populations using nuclear DNA markers (e.g. Rosel *et al.*, In press). Although microsatellites are more variable than mtDNA and have higher mutation rates rendering them more sensitive to population substructuring, they are influenced by recombination. If males are moving among geographic areas and contributing to the gene pool in adjacent areas, nuclear markers may not reveal fine-scale substructure. For example, Rosel *et al.* (In press) found no differences among western North Atlantic harbour porpoise sub-populations at six microsatellite loci, but did find differences among these same sub-populations for mtDNA. This suggests that matrilineal sub-populations exist in the western North Atlantic, with a disproportionate amount of gene flow occurring via males. Hence, basing population structure solely upon either nuclear markers or mtDNA could be misleading.

Genetic diversity and historical connections

In this study, levels of haplotypic and nucleotide diversity were similar to levels found for harbour porpoises from other areas of the eastern North Atlantic (Tiedemann *et al.*, 1996; Walton, 1997; Rosel *et al.*, 1999) and higher than those reported for the Baltic and Black Seas (Rosel *et al.*, 1995; Tiedemann *et al.*, 1996) (Table 1). Haplotypic diversity was lower than had been reported for the western North Atlantic and the Pacific (Rosel *et al.*, 1995; 1999). The lower haplotype diversity is a function of the presence of a single dominant haplotype and many rare or uncommon haplotypes. There was a predominance of a single haplotype (N1) in all regions, with the majority of the remaining haplotypes each attributable to a single individual. This pattern may be an indication of a recent rapid expansion in range on an evolutionary time scale (Avise *et al.*, 1984; Rosel *et al.*, 1999). It is possible that the modern range of the harbour porpoise had been reduced during the most recent quaternary glaciation. Up until about 16,000 years ago, the North Sea was an Arctic desert and the Baltic Sea was under ice (Adams and Faure, 1997), and thus the range of the harbour porpoise would not have encompassed its present day extension into the North Sea, Baltic Sea and Irish Sea. Approximately 11–14,000 years ago, the southern North Sea was a birch forest, but the northern reaches of the North Sea were again underwater. The Baltic Sea was still above sea level and the English Channel and Irish Sea were only partially open (Adams and Faure, 1997). At this time, porpoises could have moved into the northern part of the North Sea and the open areas of the Channel and Irish Sea.

The present day coastline is similar to conditions 9-10,000 years ago when all areas were below sea level and some channels were open to the Baltic Sea. Thus, the expansion of harbour porpoises into their modern day range in the Northeastern Atlantic is no older than 9-10,000 years, and a recent rapid expansion on an evolutionary scale could have taken place within this time frame. With an approximate generation time of 5 years there would only have been 2,000 generations since the North Sea was reopened. It is unlikely that substantial changes in molecular diversity between sub-populations in the Northeastern Atlantic could have taken place in such a short time, as approximately $4n$ generations would be required for lineage sorting to occur (Avise *et al.*, 1984). With an estimated population size in the North Sea of nearly 350,000 individuals (Hammond *et al.*, 1995), monophyly would be expected to arise within this area after approximately 1,400,000 generations (roughly 280,000 years) if gene flow were less than one individual per generation. Therefore, it would be unreasonable to expect levels of differentiation approaching monophyly within this region.

Implications for conservation and management

Although evolution of the mtDNA molecule is considered to be high (approximately 2% per million years for some animals; Hoelzel *et al.*, 1991), to some degree genetic studies are representative of historical relationships among groups, rather than current relationships (Palumbi *et al.*, 1991). Thus, the lack of significant differences in this study may be more representative of past connections between areas rather than reflective of more current relationships. In addition, the interpretation of the mtDNA data is to some extent hampered by a lack of knowledge of demography, ecology and behaviour (Aguilar and Jover, 1982; Rorvik *et al.*, 1985). Ecological studies should provide valuable insights into the current status and distribution of stocks or sub-populations. For example, Read and Westgate (1997) used satellite telemetry to map porpoise movements in the Bay of Fundy and Gulf of Maine in the western North Atlantic. Nine porpoises were tracked from 2-212 days and during this time none moved out of the Bay of Fundy/Gulf of Maine region. They concluded that the movements of porpoises in the area are restrictive enough to consider this a single sub-population.

Information on stock identity and distribution may also be obtained through the study of ecological factors (Avise, 1994) such as pollutant burdens, parasite burdens or fatty acid profiles. For example, Westgate and Tolley (1999)

found that organochlorine contaminant loads differed among porpoises from the Gulf of Maine, Gulf of St Lawrence, and Newfoundland in the western North Atlantic (see also Westgate *et al.*, 1997). The results were in agreement with the mtDNA findings of Wang *et al.* (1996) and Rosel *et al.* (In press). Although the differing organochlorine loads do not imply genetic differences, they do imply that porpoises may have long-term affiliations with particular areas. Further, radionuclide levels in porpoises from the Irish Sea were found to be significantly higher than those from the Celtic Sea or North Sea (Berrow *et al.*, 1998), suggesting ecological separation of the Irish Sea porpoises from the other areas. This compliments Walton's (1997) molecular study in which porpoises from the North Sea were found to be genetically distinct to those from the Irish Sea. As noted by Donovan (1991), information from a suite of techniques is required for a comprehensive examination of stock structure in a management context.

There are several aspects to considering conservation and management needs for harbour porpoises, and both stock structure (genetic and ecological), porpoise movements and migration rates should be assessed when considering alternatives to, or improvements upon, conservation and management plans. Although clarification of genetic differences between the North Sea Norway and Barents Sea must await further study, the differences between females from the North Sea Norway-North Sea UK and the Barents Sea-North Sea UK demonstrated in this study are sufficient to define these regions as management units (Moritz, 1994). These differences may be best described as a matrilineal population structure, and support a metapopulation model whereby overall genetic differences are larger with increased geographic distance. In addition, these results support a model for phylogenetic continuity with partial spatial separation (Avise *et al.*, 1987). In this case, one haplotype (N1) was common in all geographic regions. Many of the remaining haplotypes were both closely related to N1, and unique to one geographic region, indicating that N1 is likely to be an ancestral haplotype. Such a situation is indicative of historically intermediate levels of gene flow among the geographic regions (Avise *et al.*, 1987). Therefore, distinct lines of division are likely to be inappropriate in characterising the stock structure of harbour porpoises in the Northeastern Atlantic.

To assess the significance of bycatch rates, managers need to know if rates of replacement through population growth and migration are likely to compensate for removals. In some areas, it has been suggested that rates of population growth do not exceed removal rates (Woodley and Read, 1991; Donovan and Bjørge, 1995). Hence, managers may ask if the deficit can be made up through migration from adjacent sub-populations which do not experience high rates of bycatch. Although the number of migrants per generation (*Nm*) were estimated from the genetic data in this study, this estimate is essentially of little assistance to managers in deciding if migration rates are sufficiently high to cover yearly losses through removal. Unfortunately, uncertainties involved in estimates of effective population sizes make the translation of *Nm* into rates of exchange per year unreliable. Bycatch rates in most areas surveyed range from 1-5% per year (Woodley and Read, 1991; Jefferson and Curry, 1994; Donovan and Bjørge, 1995), but the number of female migrants per generation estimated from this study (5.5-7.7) is probably too small to compensate for high bycatch rates in affected areas. Although males may migrate in from adjacent areas, there would be no rapid colonisation and replacement by females. This is consequential because an influx of females would be necessary to increase population size through recruitment in an affected area (Avise, 1995). The low estimates of *Nm* for females suggest that migration rates should be examined in detail before assuming that migration into an affected area would assist in the recovery of that area. At this time, it may be prudent to assume that female migration is negligible in this respect, and there is little potential for recolonisation into an affected area. Therefore, the most precautionary approach would be to base the definition of management units on a fine scale, which may be female population structure as indicated by mtDNA markers. However, such a definition must also be considered in light of any information available on nuclear markers as well as information on ecological stocks and spatial movements.

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